Severe drought-influenced composition and ^{13}C of plant and soil n- alkanes in model temperate grassland and heathland ecosystems

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Abstract: Drought events are predicted to increase under future climate change. In temperate ecosystems, plants are capable of resisting drought due to their hydrophobic wax layer, in which n-alkanes are important constituents. In soils, plant-derived n-alkanes are comparatively resistant to degradation. To improve understanding of the significance of n-alkanes in plant-soil systems during a severe drought period (104 days), we investigated bulk organic carbon (Corg) concentration, total lipid extract (TLE) concentration, n-alkane molecular ratios such as average chain length (ACL), carbon preference index (CPI) and chain length ratios of different n-alkane compounds, in addition to the compound-specific isotope composition (13C) of n-alkanes in model temperate grassland and heathland plant-soil systems. Although plant communities of two (heathland) and four (grassland) species were available, only one representative species per biome was accessible for the current study. Heathland plants and soil revealed significantly higher concentrations of Corg and TLE compared with grassland. TLE and alkane composition responded quickly during the first drought phase (0 – 40 days). This indicates that plants were actively utilizing C and produced more n-alkanes in order to withstand drought, which was confirmed by increased (2 – 3‰) 13C values for n-alkanes in shoot biomass. However, during later drought phases all the parameters remained constant for plants and soils. This suggests limited change in biosynthesis and cycling of plant lipids such as n-alkanes during intense drought. Surprisingly, during the first drought phase, increased ACL and CPI ratios in soil demonstrated a rapid input of plant-derived long chain n-alkanes to soil, which was not expected due to the decadal residence time of alkanes in soil. The study enabled tracing of plant metabolic response in terms of alkane biosynthesis under different phases of drought and rapid cycling of alkanes in the plant-soil system.

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Severe drought-influenced composition and $\delta^{13}C$ of plant and soil *n*-alkanes in model temperate grassland and heathland ecosystems

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Highlights

- Drought influenced lipid composition in the plant-soil system.
- Higher lipid concentration in heathland vs. grassland ecosystem.
- *n*-Alkane composition changed in plants during first 40 days of drought.
- Soil *n*-alkane concentration increased during first 40 days of drought.
- 40 days after drought no change in *n*-alkane composition apparent.

ABSTRACT

Drought events are predicted to increase under future climate change. In temperate ecosystems, plants are capable of resisting drought due to their hydrophobic wax layer, in which *n*-alkanes are important constituents. In soils, plant-derived *n*-alkanes are comparatively resistant to degradation. To improve understanding of the significance of *n*-alkanes in plant-soil systems during a severe drought period (104 days), we investigated bulk organic carbon (C_{org}) concentration, total lipid extract (TLE) concentration, *n*-alkane

molecular ratios such as average chain length (ACL), carbon preference index (CPI) and chain length ratios of different *n*-alkane compounds, in addition to the compound-specific isotope composition ($\delta^{13}C$) of *n*-alkanes in model temperate grassland and heathland plant-soil systems. Although plant communities of two (heathland) and four (grassland) species were available, only one representative species per biome was accessible for the current study. Heathland plants and soil revealed significantly higher concentrations of C_{org} and TLE compared with grassland. TLE and alkane composition responded quickly during the first drought phase (0 – 40 days). This indicates that plants were actively utilizing C and produced more *n*-alkanes in order to withstand drought, which was confirmed by increased (2 – 3‰) $\delta^{13}C$ values for *n*-alkanes in shoot biomass. However, during later drought phases all the parameters remained constant for plants and soils. This suggests limited change in biosynthesis and cycling of plant lipids such as *n*-alkanes during intense drought. Surprisingly, during the first drought phase, increased ACL and CPI ratios in soil demonstrated a rapid input of plant-derived long chain *n*-alkanes to soil, which was not expected due to the decadal residence time of alkanes in soil. The study enabled tracing of plant metabolic response in terms of alkane biosynthesis under different phases of drought and rapid cycling of alkanes in the plant-soil system.

Keywords

Average chain length; carbon preference index; compound specific isotope analysis; EVENT I experiment; plant-soil system

1. Introduction

Temperate grassland ecosystems play a crucial role in the global C cycle (Reichstein et al., 2013; Lei et al., 2016), which has been facing increasing frequency of summer drought periods during the past few decades. In Central Europe, a higher frequency of drought periods

is predicted to occur during the most active season (April – September) of plant growth (Lindborg, 2007; Rowell, 2009). Drought has the potential to influence C cycling in the plant-soil system (Fuchslueger, 2014). Its impact on plant C uptake, biosynthesis, incorporation and fate of plant-derived compounds in soil is only fragmentarily known. This demands improved understanding of C cycling in the plant-soil system exposed to drought (Reichstein et al., 2013).

Among many important plant structural components like starch, sugars, cellulose, lignocellulose and other plant-derived compounds, the relative amount of lipids is lower. However, lipids are regarded as important plant constituents that protect plants against environmental stress such as drought and, furthermore, enable tracing of environmental stress by way of changes in their composition (Shepherd and Griffiths, 2006). The lipid constituents of cuticular wax contain a complex mixture of long chain fatty acids, aldehydes, alkanes, alcohols and ketones (e.g. Post-Beittenmiller, 1996). Among different constituent of the cuticular wax, *n*-alkanes are highly abundant compounds (Kolattukudy, 1970; Harwood and Russell, 1984). The amount of wax in plants, of which long chain *n*-alkanes are important constituents, can rapidly increase during the leaf growth phase in spring and early summer and remains constant throughout in the rest of the growing season (Tipple et al., 2013). However, other studies have revealed that the relative proportion of *n*-alkanes changes throughout the growing season (Chikaraishi et al., 2004; Chikaraishi and Naraoka, 2006), indicating that *n*-alkane production and variation depend on plant species and growth (Bush and McInerney, 2013), as well as on the environmental conditions to which the plants are exposed (Shepherd and Griffiths, 2006; Duan and He, 2011).

In theory, an increased amount of cuticular wax and, specifically, greater production of long chain *n*-alkanes would be expected under drought, because their hydrophobicity is directly related to chain length, as suggested by Shepherd and Griffiths (2006) and observed for many

plants, such as rice (Islam et al., 2009), wheat grass and oat (Jefferson et al., 1989). However, it remains unclear as to which time and stage of the plant growth and drought period the maximum concentration of *n*-alkanes can be observed and to which extent it is influenced by drought. This entails further questions, as many of the studies that describe seasonal and environmental effects such as drought on plant *n*-alkane composition analysed rather young plants, i.e. weeks to months (Tipple et al., 2013). Hence, it remains unclear how mature grassland or heathland plants would modify their *n*-alkane composition, if they were exposed to an extended drought period.

Most studies of drought effects on plant *n*-alkane composition did not include roots, although in the roots of selected species large amounts of lipids such as *n*-alkanes are produced and released to the soil (Huang et al., 2011; Gamarra and Kahmen, 2015). Therefore, the degree to which root lipids are influenced by drought is largely unknown.

In soil, a major part of lipids originates from plant input (Mucawi, 1981) through several pathways such as litter fall, deposition of abraised wax (Conte et al., 2003), as well as roots and rhizodeposits (Wiesenberg et al., 2010). Among plant-derived lipids in soil, *n*-alkanes contribute a small portion to the whole lipids, but they are part of the compounds that are characterized by comparatively slow turnover in soil (Marschner et al., 2008; Schmidt et al., 2011). Apart from plants, soil fauna and microorganism also contribute to soil lipids (Lorenz et al., 2007). Among the *n*-alkanes, long chain homologues (> C_{25}) derive mainly from plants, whereas alkanes with a shorter chain length can be attributed to soil fauna and microorganisms (Bray and Evans, 1961). Long chain *n*-alkanes in soils are comparatively resistant to degradation, as indicated by slower turnover compared with bulk C and other organic substances (Marschner et al., 2008).

Furthermore, several *n*-alkanes ratios were established (e.g. Jansen and Wiesenberg, 2017) to assess the source biomass and degree of degradation of *n*-alkanes, such as average chain

length (ACL) and carbon preference index (CPI). However, knowledge is lacking as to the impact of drought on *n*-alkane cycling in the plant-soil system and whether this can or not also be traced via such molecular ratios.

In addition to alkane composition, compound specific $\delta^{13}C$ analysis of long chain *n*-alkanes has been frequently used to improve understanding of environmental impact on the biosynthesis and cycling of *n*-alkanes in the plant-soil system (Collister et al., 1994; Lockheart et al., 1997; Chikaraishi and Naraoka, 2003). It is obvious that, if drought stress causes ^{13}C-enrichment for bulk C (Farquhar et al., 1989), biosynthesis of *n*-alkanes of plants exposed to drought should also lead to higher compound-specific $\delta^{13}C$ values of *n*-alkanes in the plants due to the coupling of the general photosysnthesis of plants and the biosynthetic isotope fractionation of lipids (Hayes, 1993; Heyes, 2001; Chikaraishi et al., 2004). However, to the best of our knowledge, studies showing the connection of increasing bulk $\delta^{13}C$ values and compound-specific $\delta^{13}C$ values of *n*-alkanes of plants exposed to drought are scarce. Furthermore, it is questionable, if and to which extent e.g. drought stress-induced higher production of alkanes might lead to change in soil $\delta^{13}C$ values of *n*-alkanes (Wiesenberg et al., 2004) despite their slow turnover on a range of decades (Marschner et al., 2008).

W have investigated model temperate grassland and heathland ecosystems that had been exposed to an extended, unprecedented drought for 104 days. Our study was guided by the following hypotheses: (i) Lipid concentration and *n*-alkane chain length increase, as do $\delta^{13}C$ values in shoots and roots under drought stress and subsequently to a minor degree also in soils exposed to drought; (ii) Greater changes would be expected for temperate grassland than for heathland ecosystems due to the greater persistence and slower reactivity of the heathland ecosystem compared with the grassland ecosystem.

2. Material and methods

2.1. Study site

The field study was conducted as part of the EVENT I experiment (Jentsch et al., 2007, 2011) at the Ecological-Botanical Garden of the University of Bayreuth, Germany [49°55‘19’ N, 11°34’55’’ E, 365 m above sea level (a.s.l.)]. The mean annual air temperature and annual precipitation were 8.2˚C and 724 mm, respectively. The upper soil (0 − 20 cm) was produced from homogenized topsoil from a nearby quarry distributed on homogenised sand from the same quarry (20 – 80 cm). The initial texture of the soil was loamy sand (820 g/kg sand, 130 g/kg silt, 50 g/kg clay). In the upper soil layer, the pH was 4.5 and in the lower layer 6.2.

The setup of the EVENT I experiment was based on a square design with a plot size of 2 × 2 m and, in total, 150 plots were exposed to several pre-treatments such as ambient control, drought, heavy rainfall and freeze-thaw cycles during the 2005 to 2010 (Jentsch et al., 2007).

All treatments had been maintained for five replicate plots. The plant community of the grassland plots was initially established as a mixed culture with *Plantago lanceolata, Holcus lanatus*, *Lotus corniculatus* and *Arrhenatherum elatius*. For heathland, a mixed culture of *Calluna vulgaris* and *Vaccinium myrtillus* was chosen.

We focused on only one annual grassland plant (*H. lanatus*) and one perennial heathland plant *(C. vulgaris)* which survived throughout the applied drought period, whereas other plant species were almost dried and disappeared quickly. Furthermore, for these two species sufficient plant material was available for all the sampling points throughout the whole growing season.

2.2. Repeated annual recurrent drought and severe drought

The experimental drought simulation on the experiemntal site consisted of two different drought experiments, (i) recurrent drought events over 5 years and (ii) a very severe drought

of > 3 months in the final year of the experiment. The control plots were exposed to ambient conditions for the recurrent drought events. The severity of the drought was determined by way of statistical extremity in a historical reference period (extreme value theory) which is independent of its effect on organisms, as described by Jentsch et al. (2007). Drought was defined as the number of consecutive days with < 1 mm of daily precipitation. Accordingly, a repeated annual drought treatment of 32 days/yr (recurrent years from 2005 – 2007) and of 42 days/yr (recurrent years from 2008 – 2010) was applied in June.

In 2011, a very severe drought was applied on all plots of the EVENT I experiment (control and drought plots). Before the severe drought was applied, all plots received 46.6 mm of water treatment from 11th to 13th May 2011 to adjust the same soil moisture content for all plots that were pre-exposed to drought and control treatments since 2005 – 2010 (Urbina et al., 2015). Due to different phenology of the plant species and local climate conditions (Automatic weather station, 2017: Botanical Garden Bayreuth), the most active part of the growing season at the EVENT site is typically between April and August (Menzel, 2003).

From 17th May to 28th August 2011, a severe drought (unprecedented) experiment was conducted, which exceeded the projected climate change scenario and lasted for 104 days. To achieve this, a large rainout shelter was constructed on a steel frame (Haygrove Tunnels Ltd., Ledbury) covering all 150 plots of the EVENT I experiment and covered with transparent polythene foil (folitec Agrarfolien-Vertriebs GmbH, Westerburg, Germany). The foil permitted nearly 90% penetration of photosynthetically active radiation (Backhaus et al., 2014). Since the beginning of the severe drought, the volumetric soil water content (vol%) was measured weekly for each plot over the course of the experiment (Backhaus et al., 2014). The volumetric soil water content dropped below the permanent wilting point (7 vol%) after 10 days (27 May 2011) of the drought phase and remained constant until the end of the severe drought (28 August 2011) period (Backhaus et al., 2014). The applied severe drought

consisted of 104 days, nearly 3.5 months of absloutely no precipitation, which was outside the calculated probabilities based on recorded German weather data (Automatic weather station 2017; Botanical Garden Bayreuth). The length of the drought period in this experiment was not intended to represent a certain future likelihood of drought duration, but actually considered as an experimental tool to identify plant response during the ongoing episode of the drought period (Kreyling et al., 2014). Hence, in order to monitor the impact of the severe drought period, the 104 days of applied drought duration was divided into three phases, i.e. drought phases I, II and III.

Drought phase I corresponded to the initial period, i.e. 0 – 40 days, when plants were still healthy and green. Drought phase II represented a strong drought, which occurred between days 40 and 70, where photosynthetic and biosynthetic activity obviously became limited, as indicated by wilting of plant leaves. Finally, drought phase III (70 – 100 days) represented the time when almost no further CO_2 uptake was possible and shoots looked almost dead (Srivastava et al., 2017b) and most plants were characterized by severe die-back (Backhaus et al., 2014).

The previously applied repeated recurrent annual drought of 100 – 1000 yr extreme drought for 5 ya (2005 – 2010) did not lead to any significant difference for C/N ratio, $\delta^{13}C$ and $\delta^{15}N$ values, and *n*-alkane composition for all plant and soil samples that were compared with ambient control pre-treatments for every individual sampling point throughout the severe drought experiment (Srivastava et al., 2017a). Therefore, for the severe drought period applied in 2011, we could use all replicate plots of drought and control pre-treatment as field replicates, leading to a total of 10 field replicates for grassland and 10 field replicates for heathland plant-soil systems.

2.3. Sample collection and preparation

Plant and soil samples were collected from the selected 20 plots from May – August 2011. The collection of shoot and soil samples was performed biweekly (such as on days 0, 12, 27, 40, 54, 68, 82, 96 and after 103 days of severe drought) from the individual plots. Samples of shoots were collected by cutting and pooling several green leaves for grassland or branches with green leaves for heathland plants and kept separately for the 10 field replicates. For collection of soil samples, an auger (length 15 cm, i.d 5 cm) was used, which was introduced 3× per plot and the aliquots were combined, keeping the 10 field replicates separate. All shoot and soil samples were oven dried at 40 °C. Roots were removed from soil samples and separated from the soil matrix with tweezers, washed with de-ionized water and oven dried at 40 °C. Soil samples were dry sieved to a particle size < 2 mm. All samples were ground in a ball mill (Retsch MM 200, Germany) to fine powder.

2.4. Bulk carbon and stable carbon ($\delta^{13}C$) isotopic analysis

Shoot, root (1 mg each) and soil (10 mg) samples were weighed in Sn capsules and analysed to determine bulk C concentration and the stable carbon ($\delta^{13}C$) isotope composition. As all samples were free of carbonate, the bulk C concentration was equivalent to organic C (C_{org}) concentration, this being relevant for the isotope values. Measurements were performed using an elemental analyser (Hekatech, Euro) coupled to a ConFlow III interface (Thermo Fisher, Bremen, Germany). Combustion of samples was followed by gas chromatography (GC) separation and transferring sample gas to a Delta V Advantage isotope ratio mass spectrometer (Thermo Fisher, Bremen, Germany). Calibration was carried out using sucrose (IAEA-CH-6, IAEA, Vienna, Austria), polyethylene (IAEA-CH-7) and the soil reference material Chernozem (Etzdorf). Within a measurement sequence, all standards were measured repeatedly together with the samples. Isotope values ($\delta^{13}C$) are expressed as per mil δ (‰) values relative to the Viennapee Dee Belemnite (V-PDB) standard and calculated on the basis of

$$\delta^{13}C = [(R_{(sample)}/R_{(standard)}-1)] \times 10^3 \quad (1)$$

where $R_{(sample)}$ is the ratio of the heavy isotope (^{13}C) to the respective light isotope (^{12}C) of the sample and the respective standard, with $R_{(standard)} = 0.0112372$ for V-PDB. Unfortunately, no isotope data were available for sampling days 12 and 96, because of a ^{13}C labelling experiment conducted on the sampled sub-plots.

2.5. Total lipid extract (TLE)

Total extractable lipids were extracted via Soxhlet extraction for shoots (*C. vulgaris* and *H. lanatus*), roots and soil samples using CH_2Cl_2/CH_3OH (93:7;v/v). Root samples represented a mixture of four grassland plants or two heathland plants, respectively. For roots and shoots 0.5 − 2.0 g were extracted and 40 − 50 g for soil. Extraction time was at least 36 h (Wiesenberg and Gocke, 2017). After evaporation of the solvent under atmospheric conditions, the TLE concentration was determined gravimetrically. Afterwards, the extract was sequentially separated into 4 fractions based on polarity.

Neutral lipid and fatty acid fractions were obtained after solid phase extraction with KOH-coated silica gel (Wiesenberg and Gocke, 2017). Neutral lipids were eluted with CH_2Cl_2 followed by fatty acids, which were recovered with CH_2Cl_2/CH_2O_2 (99:1; v:v). Afterwards, lipid fractions were dried again and neutral lipids were further separated into aliphatic, aromatic and low polarity hetero compounds using a column filled with activated silica gel (100 Å). Aliphatic hydrocarbons were eluted with C_6H_{14} followed by aromatic hydrocarbons with C_6H_{14}/CH_2Cl_2 (1:1, v:v) and low polarity hetero compounds with CH_2Cl_2/CH_3OH (93:7, v:v; Wiesenberg and Gocke, 2017). Volume reduction was performed via rotary evaporation. The sequential separation yielded four lipid fractions, of which only aliphatic hydrocarbons were studied in detail here as they contained cuticular wax components (Kolattukudy 1970;

Harwood and Russell 1984), which were expected to reveal strong compositional modification when exposed to drought (Shepherd and Griffith, 2006).

2.6. GC

A defined amount of deuteriated *n*-alkane standard (D_{50}-*n*-C_{24}) was added to the individual aliphatic hydrocarbon fractions before GC analysis. Compound identification was performed with a gas chromatograph (Agilent 6890N) coupled to a mass spectrometer (Agilent 5973N). Quantification was performed using GC with flame ionisation detection (GC–FID; Agilent 7890B). In both instruments, a J&W DB-5ms column (50 m x 0.200 mm; i.d. 0.33 µm film thickness) was used with He (purity 5.0) as carrier gas at 1 ml/min; 1µl sample was injected via an autosampler into a split/splitless injector. After a static phase of 2 min at 70 °C, the GC oven was *programmed to 320 °C (held 20 min) at 5°C/min.*

2.7. n-Alkane molecular ratios

2.7.1. Average chain length (ACL)

ACL has been used to differentiate among predominantly higher plant-derived long chain *n*-alkanes (Eglinton et al, 1962) and microorganism-derived organic matter (OM; Bray and Evans, 1961) in soils and sediments and is calculated as follows:

$$ACL = \sum(Z_n \times n)/\sum(Z_n), \quad (2)$$

where n is the number of carbons and Z_n the amount of alkanes with n carbons in the range $C_{17–33}$. High ACL values (≥ 25) derive from higher plant biomass (Kolattukudy, 1970), which is dominated by long chain *n*-alkanes (C_{25-33}; Eglinton et al., 1962). In contrast, the contribution from short chain *n*-alkanes (C_{15-23}) that derives mainly from microbial biomass

and degradation processes (Harwood and Russell, 1984) leads to low ACL values (< 25; Bray and Evans, 1961).

2.7.2. Carbon preference index (CPI)

Higher plant biomass typically shows a strong predominance of long chain odd homologous of *n*-alkanes (Eglinton et al, 1962; Kolattukudy, 1970), whereas even homologous derive mainly from degradation of OM (Zhou et al., 2005). Such a predominance of odd vs. even homologues can be expressed by the CPI:

$$CPI = [(\sum C_{23-33odd} / \sum C_{22-32\ even}) + (\sum C_{23-33\ odd} / \sum C_{24-34even})]/2 \quad (3)$$

where $\sum C_{23-33odd}$ represents the sum of the relative amounts of odd *n*-alkanes with 23-33 carbons and $\sum C_{22-32\ even}$ and $\sum C_{24-34even}$ the sum of even homologues with 22-32 and 24-34 carbons, respectively. High values (> 10) are indicative of fresh leaf biomass, while values around 1 indicate strong degradation of OM and microorganism-derived OM (Cranwell, 1981).

2.8. Compound specific isotope analysis (CSIA)

The $\delta^{13}C$ isotopic composition of individual *n*-alkanes was determined under continuous flow using a ThermoScientific Trace 1310 GC interfaced on-line via a GC-Isolink II to a ConFlow IV and Delta V Plus isotope ratio mass spectrometer. A TG-5MS column (30 m x 0.25 mm, 0.25 µm film thickness) was used. The GC temperature programme was 70 °C (held 2 min) to 320°C (held 20 min) at 5°C/min. He (purity 5.0) was used as carrier gas at a constant 1 ml/min. CO_2 of known $\delta^{13}C$ composition was automatically introduced via ConFlow IV into the isotopic ratio mass spectrometer in a series of 5 pulses at the beginning and 4 pulses the end of each analysis, respectively, and used as reference gas during every measurement.

Prior to C isotope analysis, the CO_2 reference gas was calibrated relative to V-PDB using A6 and B4 *n*-alkane mixtures provided by A. Schimmelmann (Indiana University, Bloomington, Indiana, USA) and instrument performance was routinely checked using an internal *n*-alkane standard mixture (C_{20} to C_{32}; Sigma Aldrich) with known $\delta^{13}C$ values. Calibration of these substances was done by combustion in an elemental analyser (EA Flash 2000) and measurement of the separated CO_2 in a Delta V Plus isotope ratio mass spectrometer after passing through the ConFlow IV interface (EA-IRMS). Precision for replicate measurements of the standard *n*-alkanes ranged between 0.05 and 0.08 for individual alkanes.

Isotopic values are reported as $\delta^{13}C$ values relative to V-PDB, averaging at least three replicate measurements. The averaged values of the CSIA results were used for calculation of the weighted average isotope composition of the five most abundant compounds (*n*-C_{25}, *n*-C_{27}, *n*-C_{29}, *n*-C_{31}, *n*-C_{33}) and normalized to the proportion of each:

$$\text{Weighted average of } n\text{-alkane (‰)} = (A \times \delta_A) + (B \times \delta_B) + (C \times \delta_C) + (D \times \delta_D) + (E \times \delta_E)/\sum(A{:}E) \quad (4)$$

where A, B, C, D and E represent the relative proportion of the most abundant compounds and δ_A, δ_B, δ_C, δ_D and δ_E their $\delta^{13}C$ isotopic values.

2.9. Statistical analysis

The bulk C_{org}, TLE, CPI, ACL and molecular ratios were tested for significant differences between grassland and heathland model ecosystems. Additionally, significant differences between the control (samples collected on day 0) and during the drought period were determined using Student's t-test for paired samples with a significance level of $P < 0.05$. The statistical evaluation was performed with R studio software (R development core team 2014).

3. Results and discussion

3.1. C_{org} concentration and TLE concentration

In general, the C_{org} and TLE concentration were higher for shoots followed by roots and soil for grassland as well as for heathland ecosystems (Fig. 1 and 2a). TLE concentration ranged between 9 – 16% C_{org} for shoots and decreased towards roots (4 – 9%) and soil (2 – 5%). Such patterns are a typical feature of plant lipids resulting from a large amount of plant internal and cuticular waxes (Kolattukudy 1976; Wiesenberg et al., 2012).

C_{org} for *H. lanatus* (grassland plant) and *C. vulgaris* (heathland plant) showed almost identical concentration on day 0, in agreement with previous studies, reporting C_{org} concentration in shoots ranging between 45 and 52% (Huang et al., 1997; Poorter and De Jong, 1999). TLE concentration and TLE normalized to C_{org} were significantly lower (p = 0.01) for *H. lanatus* vs. *C. vulgaris* (Fig. 2a and b). Higher TLE for *C. vulgaris* is associated with the typical features of perennial heathland plants, which often have a thicker cuticular wax layer as they usually grow under conditions with low water availability, where they use their wax layer for protection against water loss (Salasoo, 1987).

According to our first hypothesis, we expected to observe increasing TLE concentration when plants were exposed to drought. However, contrary to our expectation, TLE decreased (p = 0.05) by 28% for *C. vulgaris* and 10% for *H. lanatus* (not significant; p = 0.4) within 12 days of drought compared with the initial sampling on day 0 (Fig. 2a). This is likely related to the modification of lipid biosynthesis under drought (Laribi et al., 2009). Interestingly, during the end of drought phase I, a significant increase (p = 0.01) in TLE was observed for *H. lanatus* (61.0 ± 7.0 mg/g dry wt.) until day 40, the maximum TLE concentration for the whole observation period. TLE for *H. lanatus* decreased (21%; p = 0.05) again after 40 days and returned to the pre-drought level on day 54 (48.8 ± 2.4 mg/g dry wt.) and did not change significantly during the remaining observation period. After 54 days of drought, TLE of *C.*

vulgaris revealed the maximum value (82.0 ± 4.0 mg/g dry wt.) for the observation period, significantly higher (p = 0.01) than the value on day 0.

Afterwards, TLE did not change significantly for the rest of the drought period. The changes, especially during the first two drought phases (until day 54), indicate that, during the ongoing drought period, biosynthesis of plant constituents changed to increasing amount of hydrophobic components such as lipids related to overall biomass as an acclimatization to drought conditions (Hamrouni et al., 2001; González and Ayerbe, 2010). During drought phases II and III, lower C_{org} and TLE, as well as TLE normalized to C_{org} were observed for *H. lanatus* vs. *C. vulgaris*, in line with the expectations of our second hypothesis. Since the current study is the first to investigate the lipid composition in temperate grassland and heathland shoots during a severe drought of 104 days, the observed changes in the TLE could not be verified by other studies.

The grassland roots represented a mixture of four species and the heathland roots a mixture of two species. During sampling, it could be observed that the fibrous root systems of the grass species were closely connected to each other and produced a densely rooted depth interval in the upper 5 - 10 cm of the soil. For heathland, the roots occurred much more locally around the individual stems of the plant individuals, which is why in some soil cores only a very low amount of root biomass could be retrieved. C_{org} did not show any difference between grassland and heathland roots during drought phase I.

For roots from both ecosystems, C_{org} increased significantly (p = 0.05) during drought phase II and later decreased during drought phase III (Fig.1). However, during phases II and III grassland roots revealed significantly lower (p = 0.05) C_{org} than that of heathland except for day 82. The differences between grassland and heathland C_{org} concentration might be related to the thicker, woody tap-root tissue of heathland plants vs. the more fibrous roots of grassland species, entailing a different composition.

TLE and TLE normalized to C_{org} were significantly lower (p = 0.01) for grassland roots vs. heathland roots at the first sampling date. TLE of heathland roots decreased by 35% (p = 0.05) after 12 days of drought, whereas no change was observed for grassland roots for the same interval (Fig. 2a and b). Maximum TLE was observed for grassland roots (25.5 ± 3.5 mg/g dry wt.) on 40 days of drought. During phases II and III, TLE concentration in roots did not considerably change for both communities. In general, less variability in TLE of roots vs. shoot biomass argues for much less drought stress-related effects on biosynthesis within roots vs. shoots. This might be related to the suberin vs. cutin biopolymer structure of root vs. shoot tissue (Kolattukudy, 1981).

The C_{org} concentration for the grassland and heathland soils is consistent with the literature (de Brogniez et al., 2015). No significant difference was observed for C_{org} between grassland and heathland soils (Fig 1). C_{org} of grassland and heathland soils decreased (29%; p = 0.01) within the first 12 days of the observation period. However, during the end of drought phase I, an increase in C_{org} was observed (34%, p = 0.05) for both ecosystems.

Afterwards, C_{org} did not show significantly different values during drought phases II and III in comparison with day 0. The significantly different value of C_{org} for only one sampling (day 12) is quite surprising and uncommon in soils, as soil C pools typically do not significantly change within a few weeks. As soil lipid composition also in part revealed differences for this specific sampling date (see Fig. 2a and b) vs. other sampling dates, it is thought that, during sample collection or sample preparation, an analytical error occurred for samples for this specific day, which could not be identified. Therefore, we regard this specific sampling point with caution, but try to focus more on general trends that could be observed over several weeks.

It was anticipated there would be changes in TLE concentration for grassland and heathland soil since we observed changes in the amount of TLE in the grassland and heathland plants.

TLE concentration and TLE normalized to C_{org} were significantly lower (p = 0.05) for grassland soil than heathland soil during the whole period except for day 12 (Fig. 2a and b). Similarly, as for grassland shoots and heathland shoot as well as root samples, TLE decreased (p = 0.01) for grassland (17%) and heathland (35%) soils within the first 12 days. The grassland soil did not reveal any change in TLE concentration during drought phases II and III. For heathland soil it increased significantly (p = 0.01) on 40 days when compared with the control on day 0. Afterwards, no significant changes were observed for grassland soil and heathland soil drought phases II and III. In general, shifts in soil TLE concentration during the first drought phase and the absence of changes thereafter was in line with changes for shoot and root TLE. Further explanations of shifts in TLE are discussed below from the alkane composition.

3.2. n-Alkane molecular ratios

3.2.1. CPI and ACL

CPI and ACL have been frequently applied for source apportionment of shoot- and root-derived *n*-alkanes (Gocke et al., 2013). In general, the CPI for grassland shoot biomass is characterized by significantly (p = 0.01) higher values than corresponding root biomass (Fig. 3a). This is related to the strong predominance of long chain *n*-alkanes (C_{29},C_{31} C_{33}) in leaves, whereas roots have a lower predominance (Huang et al., 2011; Angst et al., 2016). ACL did not differ significantly between shoot and root biomass for grassland. The CPI values of grassland soil samples ranged between 5 and 8, intermediate between shoot and root biomass of the respective ecosystems (Fig.3a). For the grassland ecosystem, soil, root and shoot samples revealed identical ACL values. Thus, ACL and CPI values of the grassland ecosystem suggest that the source of the soil *n*-alkanes samples is a mixture of root and shoot biomass.

Heathland shoots showed significantly higher CPI and ACL (p = 0.01) values for shoots than

roots (Fig. 3b), confirming literature results (Huang et al., 2011; Angst et al., 2016). In general, the differences between the *n*-alkane composition of root and shoot biomass is related to the different biochemical structure of both plant tissues, with cuticular wax *n*-alkanes being more significant for leaf than for root biomass (Kolattukudy, 1981; Huang et al., 2011). The CPI and ACL values for heathland soil ranged from 5 – 10 and 29 – 30.5, respectively (Fig. 3b). The CPI and ACL values for soil samples can be directly related to the living plant biomass degradation or selective preservation of very long chain *n*-alkanes (Buggle et al., 2010; Lichtfouse et al. 1998). The ACL values of heathland soil and roots were almost identical, but lower than shoot biomass. Lower ACL values of soil than shoot biomass might indicate that not *C. vulgaris* but the other plant species on the plots (*V. myrtillus*) might contribute to a greater extent to shoot-derived *n*-alkanes in soil.

Overall, the ACL values of all sample types like shoot and root biomass as well as soil of the grassland ecosystem were significantly lower ($p = 0.01$) vs. the corresponding sample types of the heathland ecosystem. For CPI, no differences were observed between the grassland and heathland plant-soil systems. The difference in ACL is in line with heathland ecosystems and grassland ecosystems (Diefendorf and Freimuth, 2017, Jansen and Wiesenberg, 2017).

We expected to observe an increase in long chain *n*-alkane concentration in shoot biomass, after exposure to a drought period of several weeks (Shepherd and Griffiths, 2006; Kosma et al., 2009), subsequently raising ACL values. Furthermore, an increase in the production of odd long chain *n*-alkanes was also expected to increase the CPI. However, after 12 days of drought a significant decrease ($p = 0.04$) in ACL and CPI values was observed for *H. lanatus* compared with day 0 (Fig. 4a, b).

Afterwards, no significant change in ACL and CPI values was observed for grassland shoots during the remaining observation period. The decrease in ACL and CPI during drought phase

I could be a result of a specific adaptation of the investigated grassland plant, as observed for other plants (Zhang et al., 2005; Duan and He, 2011).

The ACL and CPI values did not change during the drought period for the heathland plant *C. vulgaris* (Fig. 4a, b). Such a lack of change could be attributed to the fact that no adaptation in the biosynthesis of longer chain *n*-alkanes occurred during the drought. This supports the fact that heathland plants with thick leaves and woody tissues are well adapted in terms of their alkane biosynthesis to the often water limited conditions in heathlands, which is different from grassland plants that grow under various moisture regimes (Kirkels et al. 2013; Diefendorf and Freimuth, 2017; Jansen and Wiesenberg, 2017).

ACL of grassland roots increased ($p = 0.05$) during the first 12 days of drought (Fig. 4a) and remained almost constant during later drought phases, with the exception of day 54, where a significantly higher value ($p = 0.01$) was observed vs. adjacent sampling dates. No significant shift was observed for CPI values of grassland roots during the whole observation period (Fig 4b).

Opposite to heathland shoots, CPI of heathland roots decreased with observation time (Fig. 4b). ACL for heathland roots decreased ($p = 0.01$) until day 40 of the drought phase and remained almost constant until the end of the drought Fig. 4a). The decrease in ACL and CPI in roots during drought might indicate an increased production rate of alkanes, where chain elongation is reduced and the production of by-products (Post-Beittenmiller 1996; Shepherd and Griffiths, 2006) such as even alkane homologues, leading to lowering of CPI. This overall trend suggested that production of long chain *n*-alkanes is a key response of plants against drought (Kosma et al., 2009; Guo et al., 2015).

ACL and CPI significantly increased ($p = 0.05$) for grassland and heathland soils during the first 12 days of drought and remained almost constant until the end of the drought period (Fig.

4a, b). The quick response of soil ACL and CPI values was surprising, as significant changes would not be expected in soil, because of the large alkane pool in soil that is characterized by a decadal turnover time (Marschner et al. 2008). However, likely due to plant exposure to drought stress, an increased release of cuticular alkanes as abraded wax as well as root-derived alkanes and a subsequent incorporation of these plant-derived alkanes into soil seem to be responsible for the observed change.

During later drought phases, no significant change was observed for soil *n*-alkanes. This is not surprising if we take into account that, during drought phases II and III, plant C uptake and translocation towards soil decreased significantly compared with drought phase I (Srivastava et al., 2017b). Furthermore, the direct input of plant-derived *n*-alkanes during the drought phase seemed to equilibrate degradation of soil alkanes, thereby leading to almost constant alkane composition during the remaining drought season after day 12 (Eglinton and Eglinton, 2008; Buggle et al., 2010).

3.2.2. Relative abundance of long chain n-alkanes

To obtain more specific information for source apportionment, the relative composition of most abundant plant-derived long chain *n*-alkanes (*n*-C_{25} to C_{33}) were examined (Fig. 5 and Supplementary information, Table S1). The relative proportion of the most abundant long chain *n*-alkanes has been frequently used for source apportionment of soil *n*-alkanes (Schwark et al. 2002; Huang et al., 2011; Buggle et al., 2010).

H. lanatus was characterized by an equal relative proportion (39.2 ± 3.0%) of *n*-C_{25+27}, and *n*-C_{31+33} (38.1 ± 5.4%) at day 0, which has also been already described (Bush and McInerney, 2013; Kirkels et al., 2013). *n*-C_{25+27} were the most abundant long chain *n*-alkanes of grassland roots (48.2 ± 2.3%) and soil (43.4 ± 1.0%), which showed the maximum at day 0.

Heathland shoots revealed a high proportion of *n*-C_{31+33} (85.0 ± 0.5) vs. other *n*-alkanes on day 0. Like heathland shoots, roots (58.9 ± 5.7) and soil (40.4 ± 4.7) revealed comparatively higher proportions of *n*-C_{31+33} than *n*-C_{29} and *n*-$C_{25+27.}$ A strong enrichment of *n*-C_{31+33} in heathland shoots, roots and soil is in agreement with the literature (Huang et al., 1997; Kirkels et al. 2013).

Within drought phase I (days 12 – 40), *H. lanatus* shoots showed an equal predominance (37.5 ± 3.2%) of *n*-C_{29} and *n*-C_{31+33} (37.6 ± 5.7%). During later drought phases II and III, *n*-C_{29} became the most abundant *n*-alkane in heathland shoot biomass. The changes in *n*-alkane composition are likely related to the modified biosynthesis of *n*-alkanes during drought (Kim et al., 2007; Kosma et al., 2009). Heathland shoots revealed a high relative abundance (80 – 85%) of long-chain *n*-C_{31+33} vs. other *n*-alkanes, with no significant trend during drought exposure.

n-C_{25+27} were the most abundant long chain *n*-alkanes in grassland roots (48.2 ± 2.3%) and soil (43.4 ± 1.0%), which showed a maximum on day 0 (Fig. 5 and Supplementary information, Table S1). The abundance of *n*-C_{25+27} ranged between 30 and 50% and between 25 and 50% for *n*-C_{31+33} for grassland roots during different phases of the drought. As for heathland shoots, roots also showed a relative enrichment of *n*-C_{31+33} (40 – 66%) throughout the whole observation period. Hence, heathland and grassland roots differed in their composition of long chain *n*-alkanes.

The strong enrichment of *n*-C_{25+27} in grassland soil on day 0 changed towards a predominance to *n*-C_{31+33} (40 – 55%) during drought period (Fig. 5 and Supplementary information, Table S1). This reflects a direct incorporation of plant-derived long chain *n*-alkanes in soil. The predominance of *n*-C_{31} in grassland soil during the drought period was dissimilar to *n*-C_{29} enrichment in *H. lanatus* shoot biomass. Thus, an unknown reason resulted in different

observations made for soil compared with the only investigated shoot biomass of the model ecosystems.

A clear difference was observed between grassland (dominated by *n*-C_{29}) and heathland (dominated by *n*-C_{31}) ecosystems, in agreement with previous studies (Huang et al., 1997; Van Bergen et al., 1998). Soil alkane composition of long chain homologues was almost intermediate between above-ground and below-ground plant biomass, arguing for a mixed contribution of root- and shoot-derived OM deriving from the investigated plants. However, the other plant species that contribute in lower proportion to overall plant biomass on the plots (Backhaus et al., 2014) also contribute to soil alkane composition. This led to deviating alkane composition of soil vs. shoot OM, which is also reflected in the CPI and ACL values.

3.3. Stable isotope composition ($\delta^{13}C$)

Bulk $\delta^{13}C$ of grassland and heathland plant-soil systems varied between –25.8‰ and –30.7‰ and was in a typical range for C_3 plant-soil systems (Figs. 6 and 7; Huang et al., 1997; Farquhar et al., 1989). In general, grassland shoot biomass was characterized by higher $\delta^{13}C$ values than root biomass. For soil, $\delta^{13}C$ values were slightly higher than for plants throughout the observation period. As for grassland, heathland shoot biomass revealed higher $\delta^{13}C$ values vs. root biomass, where soil revealed $\delta^{13}C$ values comparable with shoot biomass.

Overall, $\delta^{13}C$ values for heathland plant-soil systems were slightly higher than grassland plant-soil systems. Such a similarity for soil $\delta^{13}C$ values and to plant $\delta^{13}C$ values is common as soils act as integrators of the incorporated plant- and microorganism-derived OM (Wiesenberg et al., 2004, Jandl et al., 2006) of the respective ecosystems. Furthermore, seasonal changes might play a minor role for bulk C_{org} due to the comparatively short duration of the experiment of 6 yr, resulting in considerable higher portions of bulk OM deriving from

previous cultivation vs. the experiment-derived C_{org}, taking into account the decadal turnover of soil OM (Marschner et al., 2008).

The compound specific $\delta^{13}C$ values of long chain *n*-alkanes (*n*-C_{25} to *n*-C_{33}) in shoots, roots and soil of grassland and heathland ecosystems are shown in Table 1. For all sample types in the grassland they were virtually identical on day 0 (-35.9 ± 0.2 for shoots, -34.2 ± 0.5 for roots, -35.8 ± 0.3 for soil). For heathland, root and soil alkanes revealed an identical isotope composition. In general and in accord with bulk $\delta^{13}C$ values, $\delta^{13}C$ values of *n*-alkanes were higher for the samples from heathland, than for samples from the grassland ecosystem, in agreement with previous observations (Huang et al., 1997; Chikaraishi and Naraoka, 2006; Krull et al., 2006).

Weighted average values of compound specific $\delta^{13}C$ values of long chain *n*-alkanes (*n*-$C_{25\text{-}33}$) of shoot, root biomass and soil from control samples (day 0) were depleted by ca. 3 – 7‰ relative to the bulk C_{org} for grassland and heathland ecosystem (Figs. 6 and 7). A similar range of differences between bulk and compound specific $\delta^{13}C$ value has been described for leaf biomass (Collister et al., 1994; Conte et al., 2003; Eley et al., 2016), roots (Wiesenberg et al., 2004; Chikaraishi and Naraoka, 2006) and soil (Cayet and Lichtfouse, 2001, Wiesenberg et al., 2004).

An increase in bulk $\delta^{13}C$ values of plant tissue when exposed to drought was expected due to the reduced stomatal conductance and consequently higher ^{13}C values within the tissue (Farquhar et al., 1989). As the close connection of the overall biosynthesis in plant tissues and the specific *n*-alkane ratios, an increase in the compound-specific *n*-alkane $\delta^{13}C$ values was also expected (Shepherd and Griffiths, 2006). As further expected, the bulk $\delta^{13}C$ values, as well as compound specific $\delta^{13}C$ *n*-alkanes for *H. lanatus*, showed a 1.5‰ lower depletion in ^{13}C after 27 days of the drought period, compared with day 0 (Fig. 7a and b), which suggested de-novo synthesis of long chain *n*-alkanes during the early drought period. No significant

changes were observed for *H. lanatus* after 40 days vs. day 0. For *C. vulgaris*, 3‰ lower depletion in ^{13}C was observed after 40 days of the drought vs. day 0, which remained rather constant until the end of the drought experiment. Compound-specific data for plant *n*-alkanes from similar drought experiments were not available far comparison with these data.

In contrast to shoot biomass, the compound-specific $\delta^{13}C$ values of *n*-alkanes of root biomass revealed lower values by 3‰ and 1‰ for grassland and heathland roots respectively, after 27 days of drought vs. day 0 (Fig. 7b). This is just the opposite trend for bulk $\delta^{13}C$ values and might be related to different biosynthesis in root vs. shoot biomass. As the same values were observed for root samples of both ecosystems it is rather likely that either isotope fractionation occurs in precursor compounds of root alkanes during their formation or translocation from shoot to root biomass. During drought phases II and III, the $\delta^{13}C$ values remained almost constant for bulk $\delta^{13}C$ and also for $\delta^{13}C$ of *n*-alkanes until the end of the observation period. Although opposite trends have been observed for $\delta^{13}C$ of *n*-alkanes between roots and shoot biomass, both trends are caused by the drought stress of the plants.

Similar to plant biomass, we expected to observe higher $\delta^{13}C$ values for *n*-alkanes and bulk $\delta^{13}C$ in soil during the drought phase, but at a lower magnitude compared with plant samples. Bulk $\delta^{13}C$ for grassland and heathland soil was 1.5‰ higher, compared with the initial sampling date during the drought phase I (Fig. 7a). But the compound-specific $\delta^{13}C$ values of *n*-alkanes were 1.1‰ lower for heathland, whereas 0.8‰ higher values have been taken into account for grassland soil after 27 days of drought when compared with day 0 (Fig. 7b).

During the later drought phases II and III, these $\delta^{13}C$ values remained almost constant in the plant-soil systems. Especially during initial drought, lower $\delta^{13}C$ values of heathland soil were in good agreement with root isotope development during the same period, but opposite to the above-ground biomass. The simultaneous evolvement of heathland soil and root alkane isotope values might indicate that, during drought phase I, a stronger input of root-derived

alkanes occurred. As during later stages of the drought (phases II and III), neither changed for compound-specific $\delta^{13}C$ values of root or for soil *n*-alkanes for grassland and heathland soils and bulk isotope values were almost identical for both sample types, further conclusions on incorporation of root-derived alkanes could not be drawn. However, greater similarities between root and soil alkanes rather than between shoot and soil alkanes argue for a predominant root-derived source of soil alkanes here. This has been controversially discussed in the recent past, where some authors stated that roots do not play an important role in soil profiles (Schäfer et al., 2016), while others found clear evidence for the significance of root C at a molecular level (Jansen et al., 2006; Gocke et al., 2014) as well as for bulk C (Schmidt et al., 2011). Our results during initial drought support the latter, demonstrating incorporation of root-derived *n*-alkanes during the initial drought.

Overall, the increase in compound-specific $\delta^{13}C$ of *n*-alkanes during drought phase I suggested that grassland and heathland shoots were actively synthesizing long chain *n*-alkanes in order to withstand drought, supporting our first hypothesis. We observed differences, especially between root alkane $\delta^{13}C$ values of heathland, which were different from grassland. Although this is not a clear evidence for our second hypothesis, we could conclude that the investigated grassland and heathland plants obviously have different strategies for adopting their lipid biosynthesis to drought.

4. Conclusions

This is the first study describing the C and lipid composition in model temperate grassland and heathland ecosystems exposed to severe drought periods lasting 104 days. It provided evidence for an active response of lipid biosynthesis to drought stress as reflected in the TLE and *n*-alkane composition of root and shoot biomass in both plant-soil systems. The strongest changes were observed during the initial drought phase, lasting until day 40, while no further significant changes in lipid composition could be determined during the following drought

phases II and III. Furthermore, comparison of grassland and heathland plant-soil systems highlighted differences in the response of alkane compound-specific $\delta^{13}C$ values after drought exposure, especially in their root systems, which supports different adaptation of both plant-soil systems to drought stress. Finally, the changes in soil alkane composition, especially during the first 40 days of drought argue for a subsequent incorporation, especially of root-derived alkanes under drought, which has not been observed, before.

Further investigations of the effect of drought periods on lipid metabolism in plant-soil systems are required to draw general conclusions, especially due to the fact that only model temperate grassland and heathland ecosystems with only two plant species representing each community were investigated here. However, the first evidence of fast adaptation of lipid cycling in the plant-soil system to initial drought highlights the significance of rhizosphere processes, even for compound classes in soil with a slow turnover, such as alkanes.

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Figure captions

Fig. 1. Time series analysis of the effect of a severe drought on C_{org} in shoots, roots and soil of grassland and heathland ecosystems (mean ± standard error of mean given, n = 10, field replicates).

Fig. 2. Time series analysis of the effect of a severe drought on (a) TLE and (b) TLE normalized to C_{org} in shoots, roots and soil of grassland and heathland ecosystems (mean ± standard error of mean given, n = 10, field replicates).

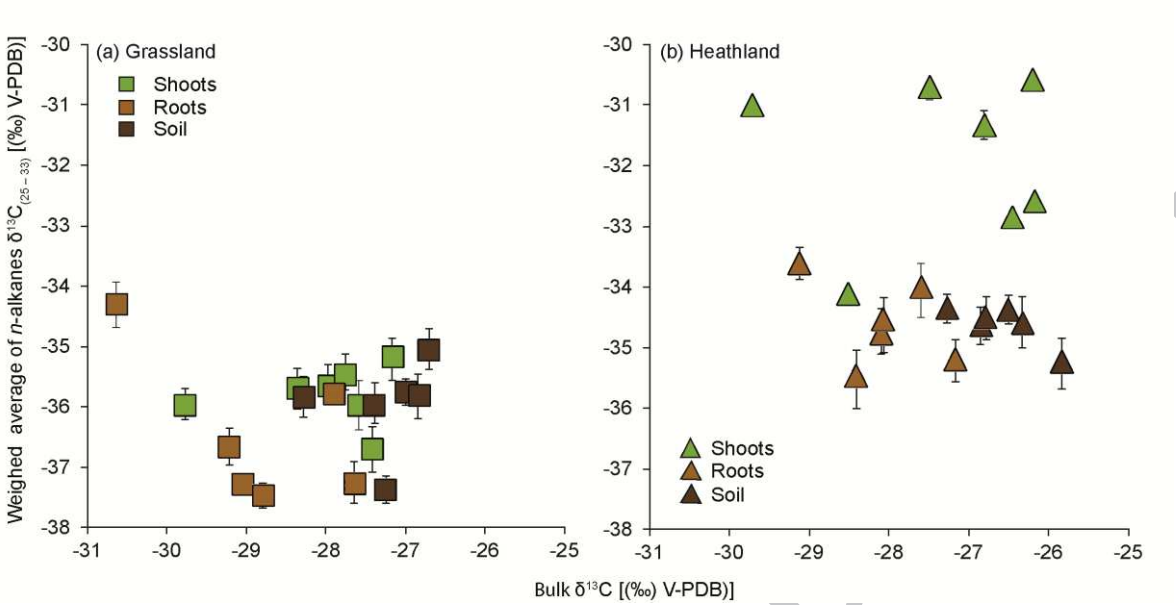
Fig. 3. CPI vs. ACL for long chain *n*-alkanes (*n*-$C_{25\text{-}33}$) showing source discrimination and apportionment in shoots, roots and soil of (a) grassland and (b) heathland ecosystems (mean ± standard error of mean given, n = 10, field replicates).

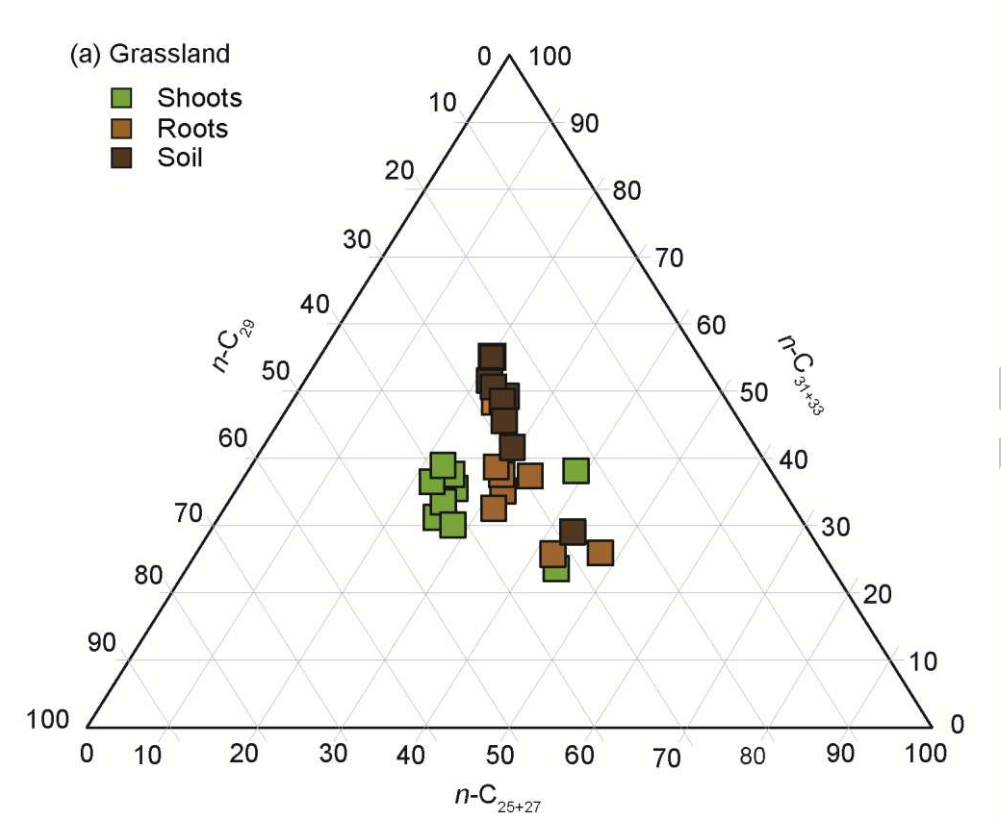
Fig. 4. Time series analysis of the effect of a severe drought on (a) ACL) and (b) CPI) of long chain *n*-alkanes (*n*-$C_{25\text{-}33}$) in shoots, roots and soil of grassland and heathland ecosystems (mean ± standard error of mean given, n = 10, field replicates).

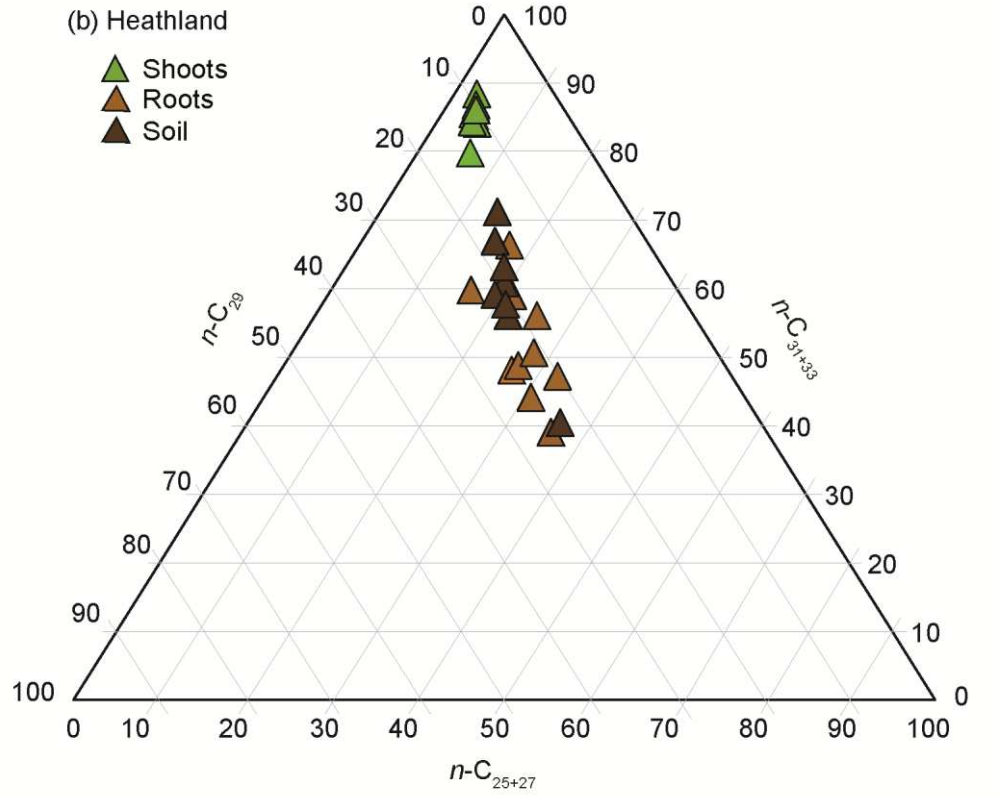
Fig. 5. Relative composition of most abundant *n*-alkanes (*n*-C_{25+27}, *n*-C_{29}, and *n*-C_{31+33}) in shoots, roots and soil of (a) grassland and (b) heathland ecosystems (n = 10, field replicates).

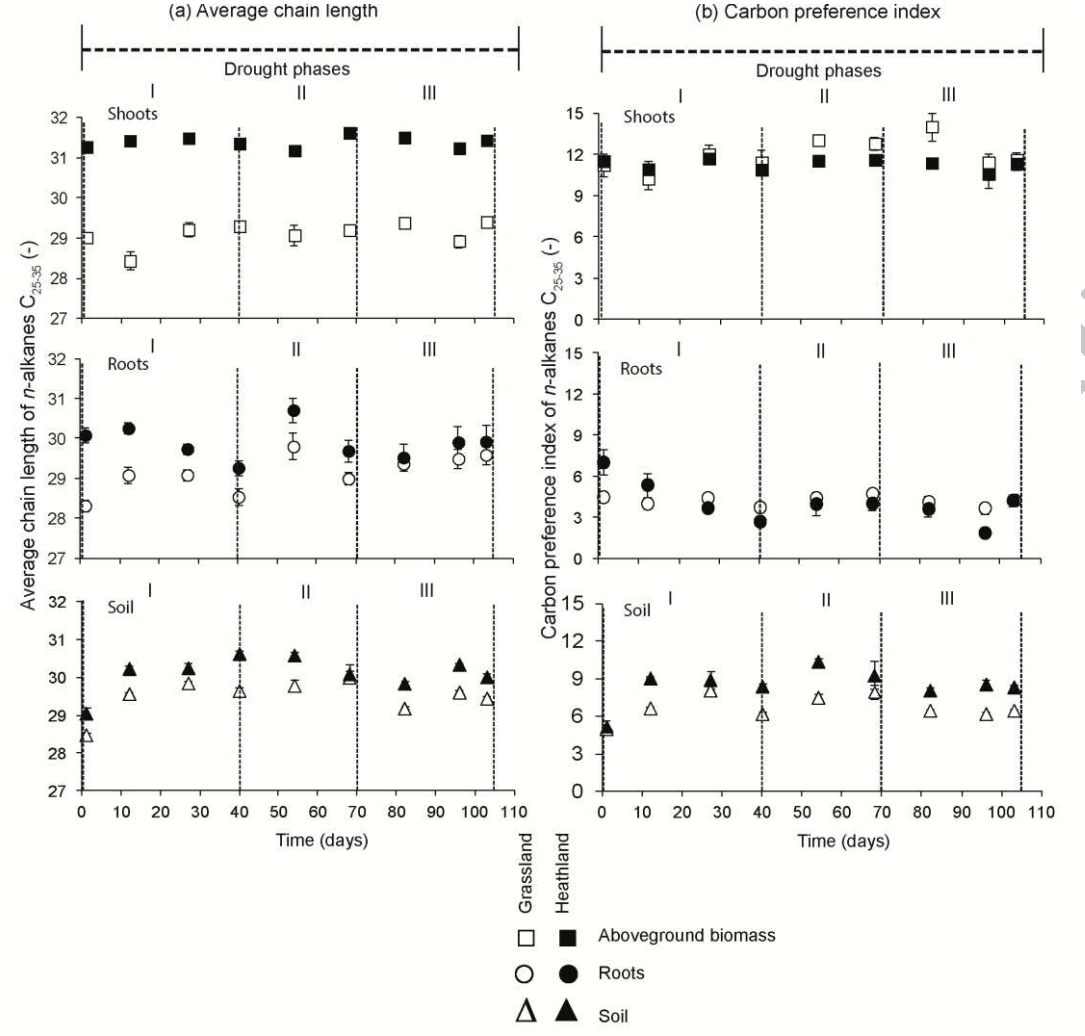
Fig. 6. Weighted average of compound-specific $\delta^{13}C$ of long chain *n*-alkanes (*n*-C_{25-33}) vs. bulk $\delta^{13}C$ isotopic composition in shoots, roots and soil of (a) grassland and (b) heathland ecosystems (mean ± standard error of mean given, n = 3, measurement replicates).

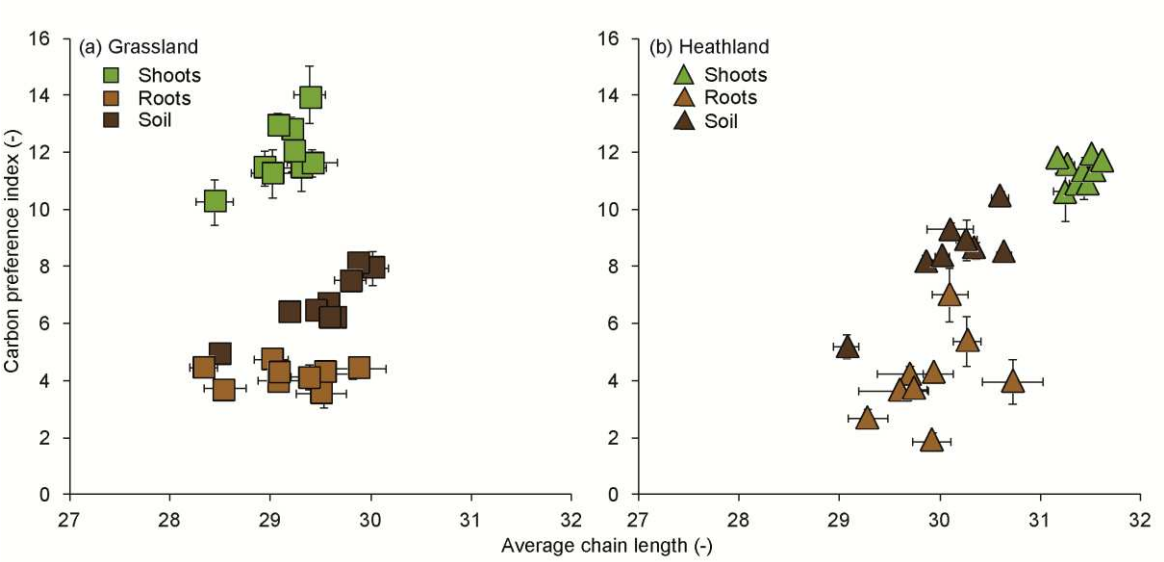
Fig. 7. Time series analysis of the effect of drought on (a) bulk $\delta^{13}C$ isotopic composition and (b) weighted average of compound-specific $\delta^{13}C$ of long chain *n*-alkanes (*n*-C_{25-33}) in shoots, roots and soil of grassland andheathland ecosystems (mean ± standard error of mean given, n = 3, measurement replicates).

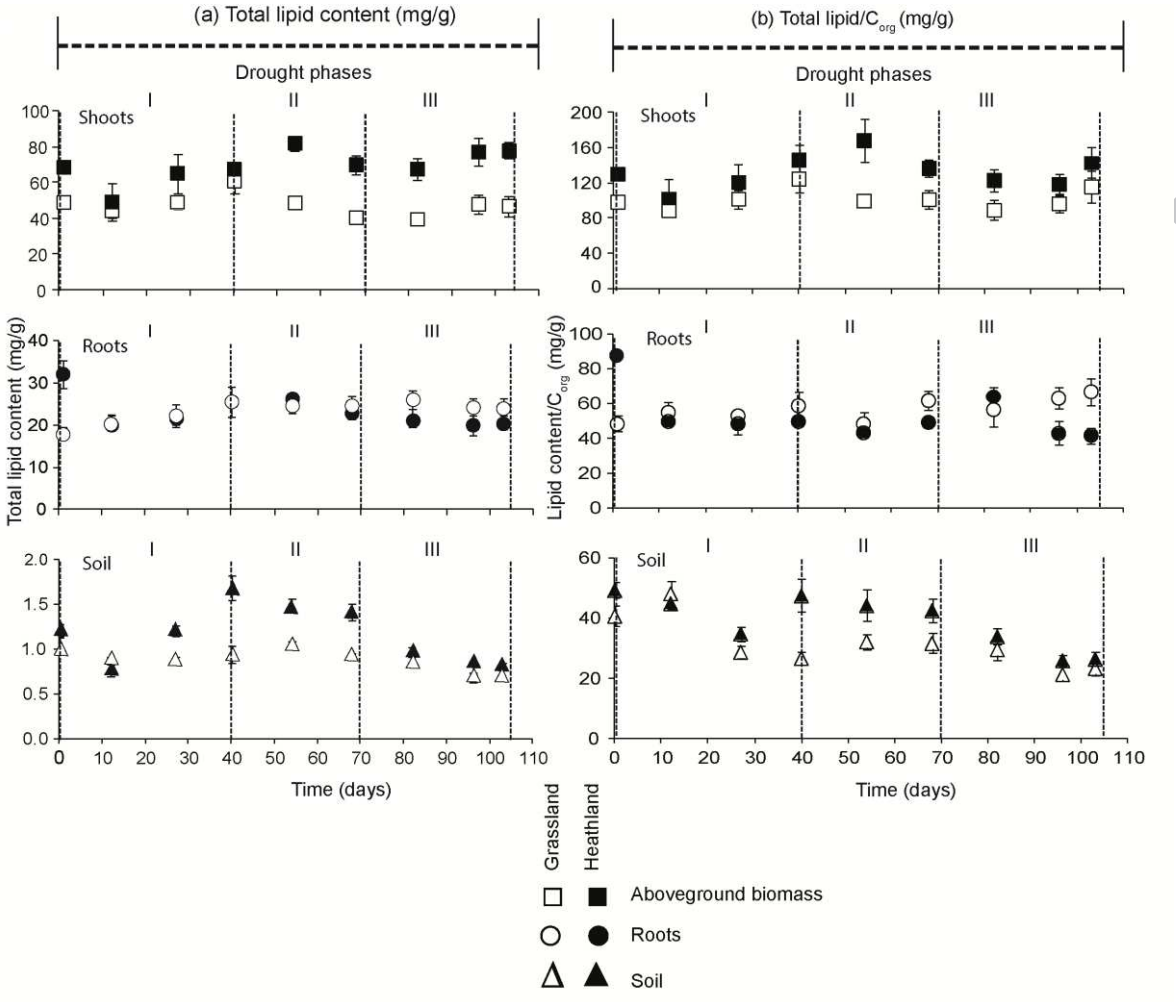


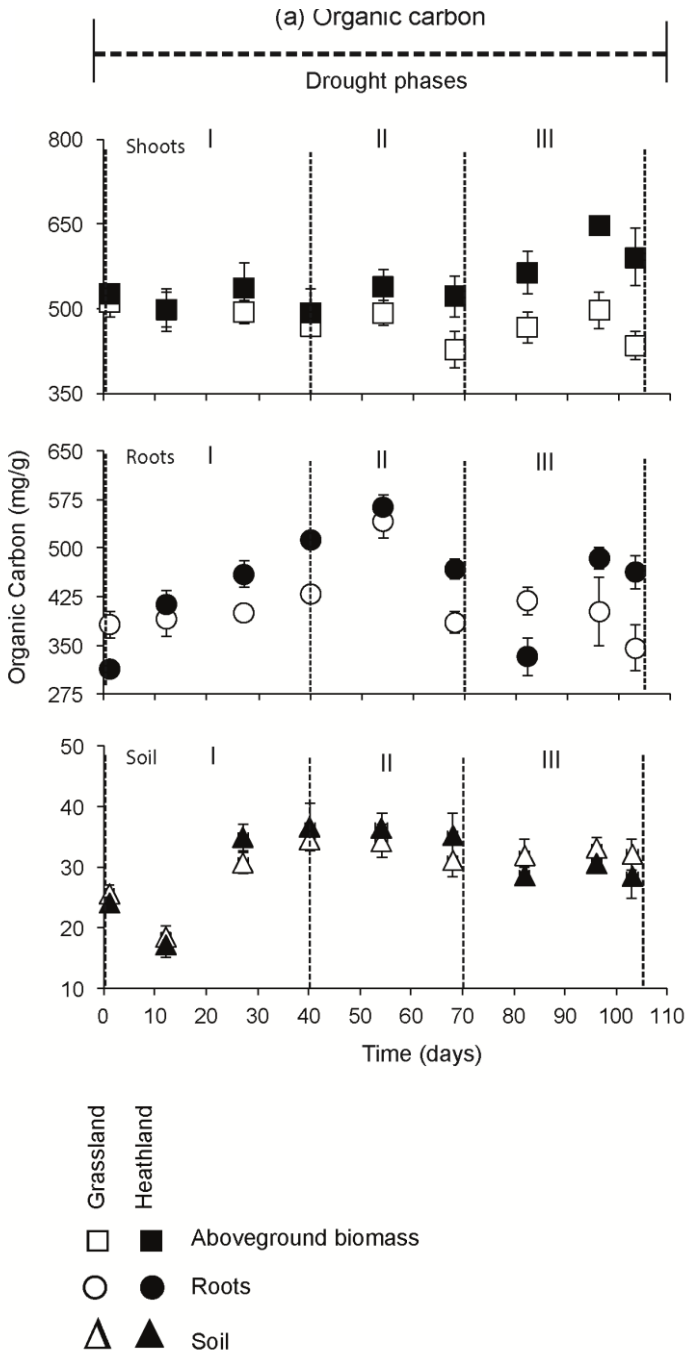

(a) Grassland
Shoots
Roots
Soil
n-C29
n-C31+33
n-C25+27


(b) Heathland
Shoots
Roots
Soil
n-C29
n-C31+33
n-C25+27









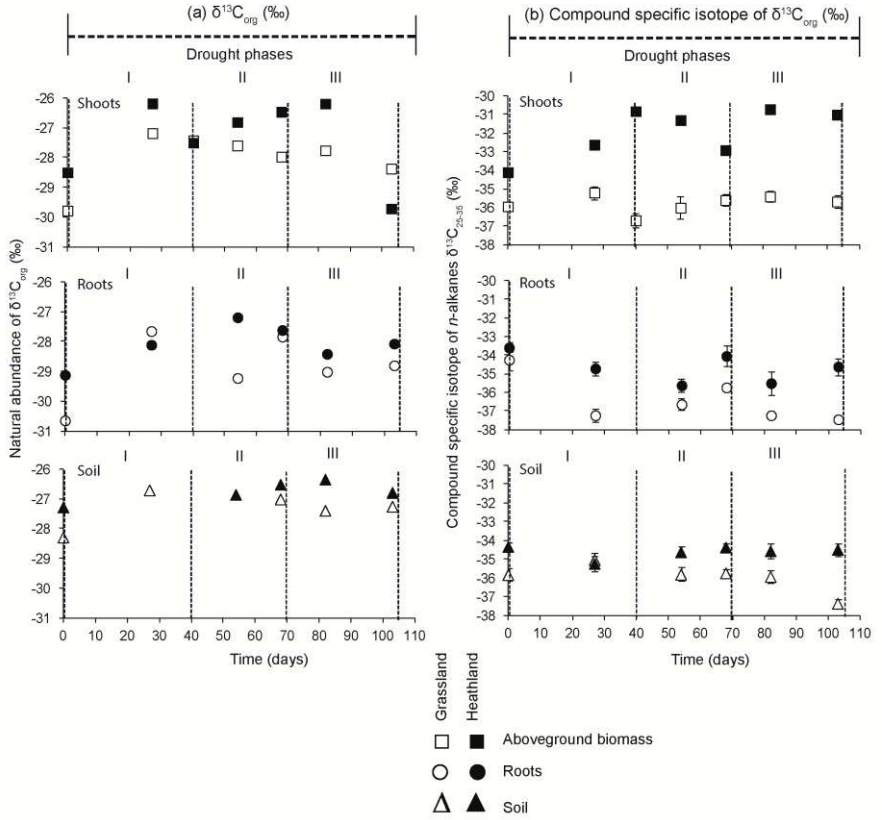


Table 1
Stable carbon isotopic composition of long chain *n*-alkanes (C_{25-33}) in model grassland and heathland ecosystems (mean ± standard error of mean given, measurement replicates, n = 3); day 0 represents control (n.a., not available).

Ecosystem	Sample	Drought phase	Sampling time	*n*-C_{25}	*n*-C_{27}	*n*-C_{29}	*n*-C_{31}	*n*-C_{33}
			(day)	(‰)	(‰)	(‰)	(‰)	(‰)
Grassland	*H. lanatus*	I	0	-34.1 ± 0.1	-35.8 ± 0.2	-36.5 ± 0.3	-36.3 ± 0.6	-36.1 ± 0.4
			27	-32.2 ± 0.3	-34.3 ± 0.2	-35.9 ± 0.2	-36.6 ± 0.1	-37.2 ± 0.1
		II	40	-33.0 ± 0.0	-35.6 ± 0.3	-37.1 ± 0.2	-37.9 ± 0.2	-37.2 ± 0.8
			54	-32.1 ± 0.0	-34.2 ± 0.0	-36.1 ± 0.0	-37.5 ± 0.0	-39.1 ± 0.1
			68	-33.4 ± 0.2	-35.2 ± 0.5	-36.4 ± 0.4	-35.8 ± 0.7	-32.9 ± 0.3
		III	82	-33.4 ± 0.4	-34.4 ± 0.1	-35.8 ± 0.2	-36.1 ± 0.2	-35.8 ± 0.1
			103	-33.9 ± 0.6	-34.5 ± 0.1	-35.9 ± 0.1	-36.5 ± 0.2	-36.9 ± 0.4
	Roots	I	0	-30.3 ± 0.0	-34.3 ± 0.1	-32.4 ± 0.1	-34.9 ± 0.1	-36.1 ± 0.1
			27	-34.5 ± 0.0	-36.3 ± 0.0	-37.3 ± 0.1	-37.6 ± 0.1	-38.2 ± 0.0
		II	40	n.a.	n.a.	n.a.	n.a.	n.a.
			54	-35.2 ± 0.4	-37.5 ± 0.5	-36.4 ± 0.4	-36.8 ± 0.8	-36.3 ± 0.4
			68	-35.4 ± 0.2	-36.1 ± 0.1	-35.8 ± 0.0	-35.7 ± 0.3	-35.5 ± 0.0
		III	82	-36.8 ± 0.2	-37.4 ± 0.3	-37.1 ± 0.1	-37.3 ± 0.1	-37.6 ± 0.2
			103	-37.2 ± 0.5	-37.8 ± 0.5	-37.5 ± 0.5	-37.4 ± 0.3	-37.0 ± 0.4
	Soils	I	0	-34.0 ± 0.2	-35.8 ± 0.1	-35.7 ± 0.1	-36.8 ± 0.1	-37.7 ± 0.1
			27	-33.2 ± 0.4	-33.4 ± 0.2	-34.7 ± 0.1	-35.5 ± 0.0	-36.4 ± 0.1
		II	40	n.a.	n.a.	n.a.	n.a.	n.a.
			54	-34.6 ± 0.5	-34.7 ± 0.5	-35.3 ± 0.3	-36.3 ± 0.2	-37.6 ± 0.4
			68	-35.3 ± 0.5	-35.7 ± 0.8	-35.6 ± 0.2	-35.7 ± 0.1	-36.1 ± 0.4
		III	82	-35.2 ± 1.0	-34.8 ± 0.6	-35.7 ± 0.6	-36.6 ± 0.3	-37.6 ± 0.2
			103	-37.4 ± 0.4	-37.8 ± 0.4	-37.6 ± 0.4	-37.3 ± 0.3	-36.3 ± 0.6
Heathland	*C. vulgaris*	I	0	-32.8 ± 0.0	-33.0 ± 0.0	-34.7 ± 0.0	-34.0 ± 0.0	-34.3 ± 0.0
			27	-32.1 ±	-32.0 ±	-32.7 ±	-32.7 ±	-32.6 ±

				0.2	0.1	0.1	0.1	0.1
		II	40	-31.1 ± 0.2	-30.7 ± 0.1	-31.1 ± 0.0	-30.8 ± 0.0	-30.8 ± 0.0
			54	-30.6 ± 0.1	-30.9 ± 0.5	-31.5 ± 0.6	-31.5 ± 0.5	-31.5 ± 0.6
			68	-32.5 ± 0.2	-32.4 ± 0.2	-33.1 ± 0.1	-33.0 ± 0.5	-32.9 ± 0.1
		III	82	-30.0 ± 0.0	-30.3 ± 0.1	-30.8 ± 0.0	-30.7 ± 0.1	-30.8 ± 0.0
			103	-31.8 ± 0.4	-31.3 ± 0.3	-31.4 ± 0.1	-30.9 ± 0.1	-31.0 ± 01
	Roots	I	0	-32.6 ± 0.4	-32.6 ± 0.2	-34.2 ± 0.2	-34.3 ± 0.4	-32.9 ± 0.6
			27	-36.4 ± 0.3	-36.5 ± 0.3	-34.7 ± 0.3	-34.0 ± 0.2	-33.2 ± 0.3
		II	40	n.a.	n.a.	n.a.	n.a.	n.a.
			54	-34.2 ± 0.2	-34.6 ± 0.0	-35.0 ± 0.1	-35.3 ± 0.0	-37.0 ± 0.1
			68	-32.2 ± 0.5	-33.0 ± 0.2	-37.8 ± 0.3	-34.0 ± 0.0	-34.5 ± 0.4
		III	82	n.a.	-32.1 ± 0.0	-36.4 ± 1.5	-36.1 ± 0.1	-36.8 ± 0.5
			103	-33.8 ± 0.3	-33.2 ± 0.0	-37.9 ± 0.2	-34.3 ± 0.0	-34.2 ± 0.2
	Soils	I	0	-32.8 ± 0.1	-33.7 ± 0.1	-34.6 ± 0.0	-35.0 ± 0.1	-35.1 ± 0.0
			27	-32.3 ± 0.1	-32.9 ± 0.0	-34.8 ± 0.0	-35.7 ± 0.1	-36.2 ± 0.0
		II	40	n.a.	n.a.	n.a.	n.a.	n.a.
			54	-32.5 ± 0.1	-32.8 ± 0.0	-34.3 ± 0.2	-35.0 ± 0.2	-35.2 ± 0.2
			68	-32.7 ± 0.0	-32.9 ± 0.1	-34.4 ± 0.1	-34.3 ± 0.0	-34.9 ± 0.0
		III	82	-32.1 ± 0.1	-32.6 ± 0.1	-34.4 ± 0.1	-35.7 ± 0.0	-35.7 ± 0.3
			103	-33.1 ± 0.3	-32.7 ± 0.1	-34.4 ± 0.1	-35.4 ± 0.2	-36.1 ± 0.2